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Biological activity of a new-synthesized polyphenol-Mg complex – preliminary data

Mihaela Mirela Bratu*, Ticuta Negreanu-Pirjol*, Lia Guiu, Sepp Porta*****

** "Ovidius" University, Constanta, faculty of Dental Medicine and Pharmacy, Romania ** "Pasteur" Institute, Bucharest, Romania *** Institute of Applied Stress Research, Bad Radkersburg, Austria Institute of Pathophisiology, University of Graz, Austria*

Using as Biopolyphenol source a lyophilised elderberry extract, a Mg complex compound was synthetized. The raw material contains: cyanidol, quercetol and kaempherol as glycosides, rutoside, chlorogenic acid, catechin and caffeic acid as bi or polycaffeoil derivatives. The magnesium source of MgO. The synthesis was accomplished at pH = 8.3 (phosphate buffer 0.067 M). The chemical analysis of the compound (FT-IR spectrum, electronic spectrum, electrical conductivity) proved that the complexation reaction did occur, since the product showed specific water-soluble compounds features. The Mg content of the complex was 5.04 mg/g. The antioxidative potential under non-biological conditions (chemoluminescence) was superior (73.4%) to the one for the wild rose fruits (64.6%), an antioxidative reference product. The effect upon the cultivated human peripheral blood lymphocytes indicates a slightly inhibitory effect on blastogenesis both for 10 mcg/mL culture concentration of the product (68.64% stimulation index) and for 50 mcg/mL culture (78.39% stimulation index).
The acute toxicity DL50 (peroral) performed on Wistar rats proved that the Mg-polyphenol complex has no acute toxicity at 2g/kg body weight and was well tolerated by the animals (no secondary effect was observed). In conclusion, the new product conserved the antioxidative properties of the Biopolyphenols. The supplementary content in magnesium could augment muscular antispastic effects and blood vessels protection, specific for polyphenols.

Key words: sambucus nigra, magnesium, complex, antioxydative activity

Highlights and drawbacks of 30 years of magnesium research: a personal view

H.G. Classen

University of Hohenheim, Fruwirthstrasse 16, 70593 Stuttgart, Germany

Mg balance is determined by uptake (usually by the oral route) and losses (e.g., faeces, urine, sweat) and regulated by enteral absorption and renal excretion. Hence, balance studies should provide reliable data for the evaluation of the actual Mg status which may be deficient, equilibrated or in surplus. Since such studies are not feasible under ordinary and clinical conditions, plasma-Mg is widely used as parameter although presenting only 0.3% of total body-Mg. In contrast to former belief, plasma-Mg is not regulated within narrow limits. Dietary factors including the Mg content of drinking water have been shown to determine the concentration of plasma-Mg in animal experiments and epidemiological studies, e.g. the Honolulu Heart Program, the Health Professionals Follow-up Study and the Women's Health Study. Epidemiological studies revealed inverse correlations between plasma-Mg and cardiovascular diseases, e.g. the NHANES-I-Follow-up Study, The ARIC study and the Framingham Heart Study as well as clinical reports, e.g. of GOTTLIEB, COHEN, BOOTH and AMIGHI. In view of these data the lower level of the reference range was increased from 0.70 to 0.76 mmol/L in 2000. Optimal concentrations are > 0.80 mmol/L and ELIN has proposed an evidence-based lower limit of 0.85 mmol/L. – Experimental studies of HENROTTE and human studies of FEHLINGER and of our group revealed subgroups of "poor Mg-utilizers" needing higher Mg-doses (than usually 15 mmol, corresponding to the RDA of adults) for the correction of hypomagnesaemia. This fact was not considered in most clinical studies and may explain poor outcome. Oral supplementation should be adjusted to increase plasma-Mg > 0.80 mmol/L. Following the first reports of SPÄTLING and of BACHEM, neuromuscular disturbances, especially benign calf cramps, are recognized as indication for Mg supplements. Magnesium is also established as "antistress-mineral" and Mg deficiency is identified as cardiovascular risk factor, generally enhancing cytotoxicity. The MAGPIE study has proven beneficial effects of pharmacological Mg doses on (pre-) eclampsia. – Underlying mechanisms include secondary electrolyte alterations (VORMANN and GÜNTHER) and interactions at excitable membra-

nes, e.g. Ca-channels (VIERLING) and the NMDA-receptor.
Main drawbacks are loss of information due to the discontinuation of Mg-specific journals, papers disregarded by databases, loss of reimbursement by public health programs and consequently less funding of studies by pharmaceutical companies, no funding of epidemiological studies in Germany and the disappearance of elctrolyte-devoted university centers. Irreplaceable are numerous deceased colleagues and friends, mentioned are Roland FEHLINGER and Joachim HELBIG.
Thirty years ago, reports on Mg were scarcely recognized by the scientific community. Today, Mg is in everybody's mouth. It is wished and hoped that research will go on successfully for personal satisfaction and human welfare.

Key words: hypomagnesaemia, prognostic value, therapeutic strategies, outlook

High prevalence of Magnesium-Depletion in patients with small-intestine-bacterial overgrowth-syndrome – Is there a correlation to fibromyalgia?

Bodo v. Ehrlich

Practice of Internal Medicine, Kempten, Germany

Rheumatologists in USA reported a significant correlation of fibromyalgia and pathological hydrogen-exhalation-test with lactulose (H2LL). Pathological test indicates bacterial overgrowth (OS). Mg was not investigated in the previous study. We reported the observation of Mg-depletion in OS patients in 1998. This gave reason for the present retrospective analysis. H2LL was mostly indicated by meteorism, intestinal talking or symptoms of irritable bowel (IBS) in part with diarrhea. Microbiological disturbances of small intestine may underly altered absorption.
Methods: H2LL performed with Quintron microlyzer in ambulatory patients. All patients with H2LL and Mgs data over 12.5 years (1992-2004) were selected (n = 526). 211 pat. with pathological H2LL (> 20 ppm H2 exhalation 60 min after lactulose ingestion) and coincident (± 4 weeks) pathol. Mgs were identified and clustered: Group A: Mgs ≤ 0.75 mmol/l; B: Mgs < 0.80 mmol/l. Patients with pathological H2LL and normal Mgs were selected as control (C).
Results: Out of 211 patients with OS 94 (f/m 3.0:1) 94 had low Mgs (A + B = 44.5%), thereof 51 (A = 24.2%) (f/m 3.5:1) pathological Ø 0.734mmol/l. 117 pat. with OS had normal Mgs Ø 0.865 mmol/l (f/m 1.54:1). Mean age A + B: f:38.3y/m:34.6y (19-69y). A positive correlation of the extent of H2-exhalation after 60 min. and the degree of Mg-depletion could not be established. A: Ø 57 ppmH2; B: Ø 73 ppmH2. Concomitant diagnosis: fibromyalgia was found in 4/94 pat. (4f/0m) with OS and Mg-depletion (Ø 0.737mmol/l) and in none of the 117 pat. with OS and without Mg-depletion. A patient with OS over 10 years and chronic Mg-depletion is described. Slight increase of diabetes (n = 3) and increased fasting blood glucose (n = 3) is observed in the group with Mg depletion.
Summary: Pat. with OS frequently have Mg-depletion. Women are concerned > 2/3, esp. young adults. Fibromyalgia correlates only with a small older subgroup ((45 y). The risk of fibromyalgia among pat. with OS + low Mgs is estimated at least 4.2%, in females at least 5.8%. Mg-depletion was found in all our patients with fibromyalgia. In patients with symptoms of IBS and fibromyalgia Mg should be monitored. We recommend the observation and normalisation of serum Magnesium in early phases of OS. Increased prevalence of diabetics and prediabetics among patients with OS is not surprising with regard of increased infection risk of diabetics and autonomous neuropathy and deserves more alert.

Key words: prescriber restrictions, magnesium, inborne magnesium depletion, diabetes mellitus, ICD

What are "inborne Mg-depletion-diseases" according to 2004 german law for patients in social health insurance – Reflections from viewpoint of internal practice

Ehrlich Bodo

Kempten, Germany

Since 2004 oral magnesium may no longer be prescribed on charge of social insurance responsible for 90% of the German population. The only exception is „inborne Magnesium depletion diseases". The legal definition suggests diagnostic certainty which is not possible in clinic and practice. Magnesium-metabolism diagnosis has to be 1. defined as clear as current possible 2. respecting that diagnostic means underlie extreme economic limitations 3. documented by means of ICD-Codes. Magnesium deficiency is presumed to be Magnesium depletion in the majority of cases. "Inborne" means either "congenital, i.e. genetically caused or connatal – intrauterine acquired". Manifestation at

birth is not part of the definition. Diabetes 2 and 1, asthma, celiac disease and migraine are magnesium depletion diseases with genetical background. Obligatory ICD code documentation should use "E83.4 – disturbance of Magnesium metabolism" + "Q99.9 chromosomal abnormality without further definition". "E 61.2 Magnesium deficiency" is optional.
A case with aortic valve prosthesis, arrythmia, and Mg deficiency demonstrates inconsistency of federal law. Intervention of scientific societies is indicated. The principle "Roma locuta – causa finita" is a risk for good patient-treatment.

Key words: fibromyalgia, small intestine bacterial overgrowth, magnesium depletion, irritable bowel syndrome (IBS), H2-exhalation-test

Emerging roles of the divalent cation channel TRPM7

Fleig Andrea

The Queen's Medical Center, 1301 Punchbowl St, Honolulu, HI 96813, USA

Based on sequence similarities, the mammalian transient receptor potential (TRP) ion channel family is divided into three subfamilies (TRPC (formerly STRPC), TRPV (formerly OTRPC), and TRPM (formerly LTRPC)). TRPM7 (LTRPC7, TRPPLIK, Chak1), a Ca^{2+}- and Mg^{2+}-permeable divalent cation channel of the TRPM channel subfamily, is required for cellular viability and has a ubiquitous distribution profile. TRPM7 is regulated by intracellular Mg^{2+} and Mg·ATP and its activity appears to be linked to cellular energy metabolism. In resting cells, physiological levels of these molecules strongly suppress the activity of TRPM7 channels and only a small constitutive activity remains, sufficient to maintain basal divalent cation fluxes. In whole-cell patch-clamp experiments, intracellular solutions that lack added Mg·ATP lead to activation of TRPM7-mediated currents that exhibit a characteristic highly nonlinear current-voltage (I/V) relationship. The large outward currents at positive potentials are carried by monovalent ions (e.g. Cs^+ or K^+), whereas the small inward currents at negative potentials are carried by divalent ions such as Ca^{2+} and Mg^{2+}. While variations in cellular Mg·ATP levels may provide an important "passive" regulatory mechanism of TRPM7, TRPM7 can be regulated by "active" mechanisms controlling the activity of these channels. Recent evidence suggests that TRPM7 activity is modulated by its own kinase domain, responding to changes in intracellular levels of cAMP induced by Gi- and Gs-coupled receptors, respectively. While the Mg·ATP-mediated regulation and the cAMP-dependent modulation of TRPM7 may be two separate and unrelated mechanisms, it is also possible that they may be linked. For example, the cAMP-induced activation of PKA could induce a shift in Mg·ATP sensitivity of TRPM7 or conversely, changes in Mg·ATP could determine the activity of PKA and TRPM7's endogenous kinase activity. Taken together, these data suggest that TRPM7's role in cellular ion homeostasis is due to its ability to integrate metabolic and receptor-mediated influences on the transport of divalent metal ions.

Key words: TRPM7, ion channel, kinase, divalent

Stable expression of claudin-16 in MDCK cells enhances paracellular and transcellular Mg^{2+} induced current

D. Günzel, J. Richter, M. Melovski, S. Amasheh, J.D. Schulzke, M. Fromm

Institut für Klinische Physiologie and Med. Klinik I Gastroenterologie, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, FU & HU Berlin, 12200 Berlin, Germany

The finding that defects in the tight junction (TJ) protein claudin-16 cause a recessive Mg^{2+} wasting disease resulting in severe hypomagnesemia and calcinosis is generally taken as indication that claudin-16 conveys paracellular Mg^{2+} and Ca^{2+} transport. To date, however, a direct proof for claudin-16 mediated transport is still missing. In the present study, we therefore investigated transport properties of the high resistance renal epithelial cell line MDCK-C7 stably transfected with claudin-16. Expression and correct localization of claudin-16 within the TJ was verified by Western blot analysis and co-localization with the TJ protein occludin in confocal micrographs of immunohistochemically stained cells.
Functional studies were carried out in an Ussing chamber set-up. Clamping transepithelial potentials to values between ± 60 mV resulted in IV-relationships that indicated increased paracellular Mg^{2+} flux in claudin-16 transfected cells. In contrast, paracellular $^{45}Ca^{2+}$ flux in claudin-16 transfected cells did not differ significantly from fluxes observed in control cells.
Surprisingly, elevated serosal Mg^{2+} concentrations caused a Na^+-dependent, amiloride-sensitive current consistent with the presence of a Na^+/Mg^{2+}-

antiport, which was enhanced in claudin-16-transfected cells. We conclude that claudin-16 directly induces a paracellular Mg^{2+} conductance and, in addition, indirectly stimulates Na^+/Mg^{2+}-antiport, which both provide pathways for renal Mg^{2+} absorption.

Key words: claudin-16/paracellin, kidney, tight junction

Magnesium supply of non-pregnant and pregnant women with different dietary regimens

T. Heuer[1], C. Koebnick[2], U.A. Heins[1], C. Strassner[1], S. Golf[3], N. Katz[3], C. Leitzmann[1], I. Hoffmann[1]

[1]Institute of Nutritional Science, University of Giessen, Wilhelmstrasse 20, 35392 Giessen; [2]German Institute of Human Nutrition Potsdam-Rehbruecke; [3]Institute of Clinical Chemistry, University of Giessen; Germany

The purpose of the present work was to examine the magnesium supply of non-pregnant and pregnant women whose habitual diets differed in the proportion of food of plant origin. The study groups consisted of 70 pregnant (VWK-S) and 239 non-pregnant women following wholesome nutrition (VWK), a diet largely corresponding to preventive recommendations, 97 non-pregnant raw food dieters (RK) consuming a diet with an amount of raw food between 70 and 100% (without beverages) as well as 35 pregnant (CG-S) and 164 non-pregnant women consuming an average Western diet (control group, CG). The non-pregnant women were 25-65 and the pregnant women 25-42 years old.

Non-pregnant women: The median magnesium intake of the VWK and the RK (515 and 530 mg/d) exceeded that of the CG (372 mg/d). The mean serum magnesium concentrations of those three study groups did not differ significantly (0.81 - 0.82 mmol/L). Magnesium concentrations in red blood cells (RBC) of the RK were 10 - 15% higher than those of the CG and VWK ($p < 0.001$, resp.). Serum magnesium concentrations of less than 0.76 mmol/L (lower reference value of the German Society for Magnesium Research) were found in 21% of the CG, 20% of the RK and 16% of the VWK.

Pregnant women: Depending on the trimester of pregnancy median magnesium intakes of the VWK-S were 468 - 504 mg/d and of the CG-S 402 - 413 mg/d. During the total course of pregnancy no significant differences in serum and RBC magnesium existed between the CG-S and VWK-S. In the morning urine of VWK-S a higher magnesium-creatinine ratio was found when compared to the CG-S ($p = 0.004$). During the last trimester, the occurrence of calf cramps was lower in the VWK-S than in the CG-S (37 vs. 64%; $p = 0.025$).

It appears that in non-pregnant and pregnant women plant-based diets are associated with higher magnesium intakes than with a Western diet. However, this is reflected only partly in serum and RBC magnesium concentrations. In the pregnant women the higher urinary magnesium excretion of the VWK-S indicates a more favorable magnesium supply with a plant-based diet. This is supported by the lower occurrence of calf cramps in the VWK-S.

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Key words: magnesium, women, pregnancy, dietary regimens

Effect of magnesium on electrically and pharmacologically induced neurotransmitter release

S. Jost, W. Vierling

Institut für Pharmakologie und Toxikologie der Technischen Universität München, Biedersteiner Str. 29, 80802 München, Germany

Inhibition of neurotransmitter release can have a positive effect in several diseases. For example, inhibition of the release of noradrenaline (NA) may reduce cardiac arrhythmias and improve the prognosis of heart failure.

It is known that magnesium can inhibit the release of neurotransmitters (e.g., acetylcholine in neuromuscular junction). Therefore, we investigated whether Mg can suppress NA release. For the experiments, we used the rat ductus deferens which is strongly innervated by sympathetic fibres. Electrical stimulation of this preparation induces contraction of the smooth muscle mediated by the release of NA. We induced the contraction by electrical stimulation or addition of NA or tyramine. ù-conotoxin GVIA, a specific blocker of neuronal (N-type) Ca channels blocked the electrically induced contraction but didn't influence the contraction induced by NA or tyramine. In contrast, Mg diminished the contractions under the influence of electric stimulation, NA and tyramine. However, the suppressing effect on electrically stimulated

contractions was much stronger than on NA- or tyramine-dependent contrations.
The results show that Mg exerts a stronger effect on neurotransmitter release than on the function of the smooth muscle. The tyramine-induced, indirect transmitter release seems not to be influenced by Mg.

Key words: magnesium, noradrenaline release, ductus deferens

Magnesium and pulse pressure in essential hypertension

K. Kisters[1], O. Hoffmann[1], B. Gremmler[2]

[1]Medical Clinic I, St. Anna-Hospital, 44649 Herne, Hospitalstr. 19, [2]Cardiological Department, Marienhospital Bottrop, Germany

The influence of a magnesium deficiency in the development of primary hypertension has often been described. However, several investigations were performed in human blood cells or smooth muscle cells in hypertension. Possibly a disturbed calcium/magnesium antagonism, reduced ATPase activities or disturbed magnesium channels are responsible for this magnesium deficiency. The height of pulse pressure (difference between systolic and diastolic blood pressure values) is known to be a risk factor for cardiovascular morbidity and mortality. In this context, it was of interest to study magnesium serum concentrations in essential hypertensives with pulse pressure > 60 mmHg and < 50 mmHg (ten patients in each group). Our results show that in the group with a high pulse pressure serum magnesium concentrations were measured 0.72 ± 0.1 mmol/l *versus* 0.88 ± 0.07 mmol/l in essential hypertensives with a pulse pressure < 50 mmHg ($p < 0.05$, r – 0,73).
In conclusion the presented data show that in patients with elevated pulse pressure values a magnesium deficiency may occure. The magnesium deficiency significantly correlates with the height of pulse pressure. The results a similar to previous investigations in essential hypertensives. As a benefit of a magnesium supplementation has been well documented in the treatment of primary hypertension, further studies are necessary to demonstrate a positive effect of a magnesium therapy in reducing pulse pressure values.

Key words: magnesium, hypertension, pulse pressure

Increased Calcium and decreased Magnesium Concentrations and an increased Calcium/Magnesium Ratio in SHR *versus* WKY.

K. Kisters[1], F. Wessels[1], E.R. Krefting[2], B. Gremmler[3]

[1]Med. Clinic I, St. Anna-Hospital, 44649 Herne, Hospital-Str. 19, [2]Med. Physics University of Münster, [3]Med. Clinic, Marienhospital Bottrop, Germany

Alterations in the metabolism of calcium and magnesium have been implicated in the pathogenesis of primary hypertension. Calcium influx across the external cellular membrane in smooth muscle cells and cardiomyocytes plays a crucial role in the control of cellular excitation contraction and impulse propagation. Intracellular calcium and magnesium concentrations are controlled by reversible binding to specific calcium binding proteins. The calcium and magnesium flux across the external membrane is regulated by a calcium pump (calcium-magnesium-ATPase), calcium channels and binding to the membrane. In cell membranes and in lymphocytes of essential hypertensives our group showed increased calcium and a decreased magnesium and increased calcium/magnesium ratio in hypertensive cells.
In this context, in aortic smooth muscle cells from 13 spontaneously hypertensive rats (SHR) of the Münster strain (systolic blood pressure 188.4 ± 9.8 mmHg) and 13 normotensive rats (NT, systolic blood pressure 118.5 ± 7.2 mmHg) aged 9 months, the intracellular calcium and magnesium contents were measured under nearly in-vivo conditions by electron-probe microanalysis. Measurements were performed in aortic cryosections 3 µm thick. The calcium content was 124.7 ± 4.5* mmol/kg dry weight in SHR *versus* 110.3 ± 4.1 mmol/kg dry weight in NT (Means ± SD, $p < 0.01$), the magnesium content was 35.5 ± 3.9* in SHR *versus* 50.1 ± 4.9 mmol/kg dry weight in NT/$p < 0.01$). The calcium/magnesium ratio was significantly increased in SHR *versus* NT (3.56 ± 0.39* *versus* 2.23 ± 0.27, $p < 0.01$).
In hypertensive one month old animals the increase in the calcium/magnesium ratio was not as pronounced as in 9 months old animals. The calcium/magnesium ratio was measured 3.3 ± 0.42 in SHR ($n = 8$) as compared to 2.51 ± 0.39 in normotensive animals ($n = 8$, $p < 0.01$).
Aortic smooth muscle cells from SHR are characterized by a markedly elevated intracellular calcium and decreased intracellular magnesium contents

compared with normotensive cells. Cellular calcium and magnesium handling is disturbed in SHR aortic smooth muscle cells as it is in hypertensive blood cells. The increased calcium/magnesium ratio in hypertensive cells is a pathogenetic factor for the development of arteriosclerosis and hypertension.

Key words: magnesium, calcium, SHR, hypertension

Recent advances in molecular genetics of hereditary magnesium losing disorders

Martin Konrad

University Children's Hospital, Freiburgstr., CH-3010 Berne, Switzerland

Recent advances in molecular genetics in hereditary hypomagnesemia substantiated the role of a variety of genes and their encoded proteins in human magnesium transport mechanisms. This knowledge on underlying genetic defects helps to distinguish different clinical subtypes and gives first insight into molecular components involved in magnesium transport. By mutation analysis and functional protein studies, novel pathophysiological aspects were elucidated.

During the last four decades, numerous reports concerning inherited magnesium losing disorders have been published and their distinctive phenotypic features have been intensively discussed. Whereas intestinal magnesium absorption is still poorly understood, phenotypic characterization of clinically affected patients and experimental studies of appropriate animal models have contributed to a growing knowledge of renal magnesium transport mechanisms. The identification of the most likely affected nephron segments in the kidney, the presentation of different modes of inheritance and the observation of additional characteristic symptoms promoted a classification into different subtypes of inherited magnesium losing disorders.

In general, primary magnesium wasting disorders are relatively rare. The prevalence of the more frequent entities, for example Gitelman and Bartter syndrome, has been estimated to be only approximately 1:50.000. For most of the other disease entities, relatively few cases have been reported in the literature. Depending on the genotype, the clinical course is sometimes mild or even asymtomatic. Therefore, the disease prevalence might be underestimated for some of these syndromes. Several reports have demonstrated latent hypomagnesemia in a high percentage in the general population, e.g. 14.5% in a recent German study. Furthermore, associations of hypomagnesemia with common chronic diseases have been reported. Hypomagnesemia is frequently detected in patients with diabetes mellitus type II, arterial hypertension, coronary heart disease, or asthma bronchiale and diminished magnesium is commonly related to an aggravation of these diseases.

Magnesium transport has been intensively studied in humans and various animal models leading to accepted concepts underlying the pathophysiology of the different forms of hypomagnesemia. However, the electrophysiological characterization of magnesium pathways has been complicated by unintentional simultaneous measurement of other cations so that the molecular correlates mediating mammalian magnesium transport components remained undefined.

A different approach to study components of magnesium transport arises from genetic analysis of families affected with magnesium wasting diseases. Linkage disequilibrium studies on affected kindreds have enabled the chromosomal localization of several genes involved in hereditary hypomagnesemia and in the last decade, a number of genes have been identified by positional cloning. These genes have provided first insight into mammalian magnesium transport molecules. This talk will focus on the most recent research as it adds considerably to our understanding of renal magnesium conservation.

Key words: hypomagnesemia, genetics, TRPM6, kidney

Hereditary Magnesium-Deficiency Tetany – a Magnesium-losing disorder

D.-H. Liebscher

SHO Mineralimbalancen e.V., Rummelsburger Str. 13, D-10315 Berlin, Germany, www.magnesiumhilfe.de

The magnesium-deficiency tetany is based on a recognized magnesium deficiency, characterized by special symptoms excluding other diseases, sometimes by magnesium serum values in the lower range of the reference values as well as by a successful Mg-loading test, it means by a therapeutical substitution test with a daily amount of 600 - 1800 mg Magnesium.

It was the convinction of Fehlinger (1991), that the so called latent tetany i.e. the magnesium-deficiency tetany of the adults has a hereditary or familiar cause.

Fehlinger has given the important hint, that 2/3 of the children of the tetanic mothers show tetanic symptoms as well. In 1990, he supported the foundation of the self-help organisation of mineral imbalances, which brings together some affected families. These families are ready for genetic investigations, so that high-risk groups of future generations can be diagnosed at an early stage by corresponding genetic tests, and in case of a positive test, they can receive lifelong oral magnesium substitution as early as possible. For this early diagnosis determines the state of health of these patients: either a long time of suffering with early retirement or a relatively active life.
With the families that have been already diagnosed, these genetic tests are not necessary for to justify the magnesium-losing disorder.

Key words: hereditary magnesium deficiency, tetany, magnesium-losing disorder, self-help organisation

Determination of Mg^{2+}, Na^{+}, K^{+}, Ca^{2+} ions in erythrocytes using ion-selective electrodes

A. Malon, M. Maj-Zurawska

Warsaw University, Department of Chemistry, Pasteura 1, 02 093 Warsaw, Poland

Nowadays ionized Mg^{2+}, K^{+}, Na^{+}, Ca^{2+} concentration in erythrocytes is determined by comparatively expensive methods requiring specialized staff. These methods are: the nuclear magnetic resonance spectroscopy (NMR), the fluorimetry using video-microscope, ion-selective microelectrodes or the zero-point titration using the atomic absorption spectrometry (AAS). That is why accurate, reproducible and cheap method for the measurement of erythrocytes Mg^{2+}, K^{+}, Na^{+}, Ca^{2+} ions concentration was required.
Ion-selective electrodes (ISEs) are successfully used for various samples, especially body fluids as blood or urine, but problems can arise if measurements concern ion concetration inside cell. In this case, the volume of the sample is small, the composition of the sample may change fast and certain ionic component interferes with the ISE being used.
However, ion-selective electrodes with improved detection limit and better selectivity, and appropriate preparation of the sample overcome all this impediments. A simple, rapid and accurate method of determining physiologically relevant cations in erythrocytes using ion-selective electrodes for routine clinical use was investigated. Potentiometric measurements were made using a clinical analyzer Microlyte 6 (Kone, Finland) in erythrocytes after lysis. The results obtained from potentiometry and reference methods (NMR, AAS, AOS) did not differ significantly.

Key words: erythrocytes, magnesium, potassium, sodium, calcium, ion selective electrodes

Measurement of ionised Mg^{2+} and Ca^{2+} concentrations, apparent equilibrium constants and net fluxes with Mg^{2+}/Ca^{2+}-macroelectrodes

J.A.S. McGuigan, J. Kay, H. Elder

University of Glasgow, Scotland, UK

Macroelectrode measurement of ionised Mg^{2+} and Ca^{2+} concentrations ($[Mg^{2+}]/[Ca^{2+}]$) in the μmolar/nmolar range requires calibration in buffer solutions. Because calculated apparent equilibrium constants (Kapp) vary and ligand purity is less than 100%, measurement of these two parameters in the buffers is necessary. The most exact method to calculate both parameters from the electrode potential measurements is that of Lüthi *et al.* (Exp. Physiol. 82, 453, 1997) but these calculations are time consuming, limiting the use of the method. This problem has now been overcome by developing an Excel program for the calculations. The program also extends the method to measure the Kapp for $[Mg^{2+}]/[Ca^{2+}]$ binding to physiological organic anions (Kay *et al.* 2004, J. Physiol. Proc., in press). The program allows rapid macroelectrode calibration and consequently the measurement of $[Mg^{2+}]/[Ca^{2+}]$ in the μmolar/nmolar range, making routine on-line net flux measurements feasible. The extension of the method means that the Kapp of the $[Mg^{2+}]/[Ca^{2+}]$ binding to organic anions under physiological conditions can be easily measured with macroelectrodes.

Key words: Mg^{2+}/Ca^{2+}-macroelectrodes, Mg^{2+}/Ca^{2+}-buffers, Mg^{2+}/Ca^{2+}-buffer calibration

The possible role of Mg ions in the treatment of tinnitus caused by direct damages of hair cells

H. Nguyen-Duong

Univ. of Ulm, 89069 Ulm, Germany
<hoang.nguyen-duong@medizin.uni-ulm.de>

A rational tinnitus therapy requires a precise localization of the cochlear damages directly related to

the perceived noise. Damages induced by noise of too high intensity or by ototoxic substances and caused by destruction and/or degeneration of inner hair cells, are accompanied by an elevated synaptic glutamate release. The resulting excitotoxicity induces *via* an excessive stimulation of postsynaptic NMDA receptors an oversensitivity of neurons of the central auditory pathway, perceived as a pathologic noise reminiscent of phantom pain. A logical therapeutic strategy would be to damp the postsynaptic NMDA receptor *via* an adequate pharmacologic modulation. It is well-known that NMDA-receptor associated ionic channels, localized on normal polarized cell membranes, are blocked potential dependently by external Mg, allowing thus a coordinated function of the signal transduction chain. Chronic hypomagnesemia together with partial depolarization of the cell membrane caused by impairment of the energy metabolism abolish the protecting blockade of the NMDA receptors by external Mg. A subsequent uncontrolled Ca Influx activates Ca dependent enzymes and produces cytotoxic free radicals, which eventually triggers the neuronal cell death. Therefore a successful strategy for therapying tinnitus caused by hair cells degeneration might be accomplished by blocking the NMDA-receptors through antagonists (such as memantine or caroverine) or by reducing the ionic conductance of the NMDA receptors associated ionic channels *via* an increase of the extracellular Mg concentration (e.g. by cochlear micro-infusion of magnesium salts), lowering thus the excitability of auditory nerve fibers.

Key words: tinnitus, hair cells damages, magnesium ions, NMDA-receptors

Involvement of magnesium homeostasis in depression and antidepressant therapy

G. Nowak[1,2], E. Poleszak[3]

[1]Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, 31-343 Krakow, Poland; [2]Collegium Medicum, Jagiellonian University, Krakow, Poland; [3]Medical University, Lublin, Poland

Antidepressant therapy exhibits low clinical efficacy and produces a variety of unwanted side effects. Thus, search for better drugs are continuously in progress. Recently antidepressant-like properties of antagonists of the NMDA/glutamate receptors have been demonstrated. Magnesium (Mg) is one of the natural antagonists of the NMDA receptor. In forced swim test (FST), a rodent test with high predictivity of antidepressant activity in human depression, Mg caused a dose dependent antidepressant-like effect (reduction in immobility time). Moreover, Mg enhances the antidepressant effect of imipramine in this test. On the other hand, deficiency of Mg ion has been related to depressive disorders. Several groups demonstrated that clinical depression might be accompanied with lower serum Mg concentrations. The results indicate that Mg produces antidepressant effects in rodents and suggest the involvement of this ion in human depression.

Key words: magnesium, antidepressants, depression

Knock-down of the Mitochondrial MRS2 Gene is Lethal in Human Cell Culture.

M. Piskacek, J. Weghuber, R.J. Schweyen

Max Perutz Laboratories, Vienna Biocenter, University of Vienna. Dr. Bohr-Gasse 9, A-1030 Vienna

The human genome encodes a single gene for the major mitochondrial Mg2 + channel protein Mrs2p. In HEK-293 cultured cells we have stably established a vector for siRNA directed against MRS2. This siRNA caused degradation of MRS2 mRNA (= RNA interference) and resulted in early death of transfectants. We have then chosen to express this siRNA from a doxicycline-regulated promoter. This resulted cell cultures which were viable in the abscence of the doxicycline but died some time after the addition of the inducer. Regulated knock-down of MRS2 was confirmed by cDNA analysis. Mitochondria isolated from cells without and with induction of siRNA revealed that the knoch-down of MRS2 resulted in a strong reduction in Mg2 + uptake capacity. Work is in progress to see whether the reduction in mitochondrial Mg2 + concentrations initiates apoptosis or results in necrosis of HEK-293 cells.

Key words: mitochondria, mutation, Mg2 + influx

Low preoperative serum Mg levels are reliable co – predictors for post operative liver failure

S. Porta, B. Bacher, H.J. Mischinger

Institute of Applied Stress Research, Bad Radkersburg, Univ. Dep. for Gen. Surgery, Institute of Pathophysiology, Med. Univ. Graz, Austria

Introduction, material and methods: 26 Patients before and during hepatectomy were investigated. Pre- peri- and postoperative serum parameters, indicative for postoperative liver failure were determined, like free plasma epinephrine, serum calcium, serum magnesium (NOVA CCX), cholesterol and triglycerides in patients with and without post operative liver failure. Statistical evaluation has been done with Clinical Stress Assessment software.
Results and discussion: In patients with postoperative liver failure, perioperative catecholamines were 3-fold ($p < 0,01$)) higher than in patients without liver failure. Preoperatively already, significantly lower triglyceride, cholesterol and – most importantly – serum magnesium values in liver failure patients were evident. Highly significant correlations between perioperative Ca and Mg (invers) values could be interpreted as increasing catecholamine influence upon Mg turnover. Such unproportional catecholamine effects preoperatively and perioperatively in the LV group, consistent with metabolic exhaustion, further increased by surgery stress, manifesting itself typically in comparatively low Mg, (Cadell, Porta), and "pseudonormal" cholesterol and triglyceride values.

Key words: magnesium, liver failure, hepatectomy, catecholamines, triglycerides, cholesterol

Postspinal headache and magnesiumconcentrations in cerebrospinal fluid, erythrocytes and plasma

P. Saur, G.-G. Hanekop, F.B.M. Ensink, D. Kettler

Georg-August-University Goettingen, Department of Anesthesiology, Emergency- and Intensive-Care-Medicine, 37099 Goettingen, Germany

Similar symptoms of migraine and postspinal headache suggest a common pathophysiological correlate. Magnesium concentrations in cerebrospinal fluid have been found significantly low in migraine patients. The study was done in order to investigate for the first time whether there is an association between postspinal headache and magnesium concentrations in cerebrospinal fluid, plasma and erythrocytes.
Twohundredfourteen (75 women, 139 men) subjects participated in the study. We included patients who were treated with spinal anesthesia because of an operating procedure. Total plasma, cerebrospinal fluid and intracellular magnesium concentrations were evaluated. The kind and size of needle and the amount of infused magnesium were documented.
6,1% (2 women, 11 men) of the 214 patients had an postspinal headache. Total plasma, cerebrospinal fluid and intracellular magnesium concentrations were not different between the patients with and without postspinal headache. The concentrations of magnesium in cerebrospinal fluid of male patients with postspinal headache who were younger than 45 years were higher than the concentrations of magnesium in cerebrospinal fluid of male patients with postspinal headache who were older than 45 years.
Further studies are needed to confirm the results of this investigation.

Key words: headache, magnesium, cerebrospinal fluid

Magnesium and Cerebral Ischemia

R. Schmid-Elsaesser

Department of Neurosurgery, Ludwig-Maximilians-University, 81377 Munich, Germany

Cell death after cerebral ischemia is mediated by a massive release of excitatory amino acids, generation of free radicals, and – a crucial step – calcium influx into cells. Magnesium (Mg) is a naturally occurring calcium antagonist that may exert beneficial effects on the ischemic brain *via* multimodal mechanisms. Mg ions (1) participate in voltage-sensitive blockade of ion channels, resulting in non-competitive antagonism of N-methyl-D-aspartate (NMDA) receptors, (2) compete with extracellular calcium ions to reduce calcium entry into cells, (3) inhibit the release of intracellular calcium ions and excitatory amino acids, (4) suppress cortical spreading depression and anoxic depolarizations, (5) presynaptically potentiate adenosine, (6) relax vascular smooth muscle resulting in vasodilation of

large and small vascular beds and increased cerebral blood flow, (7) antagonize endothelin-1 and other vasoconstrictors, and (8) replete an underlying and/or ischemia-induced Mg deficient state. Preclinical studies have shown that Mg is neuroprotective in models of focal and global ischemia, subarachnoid hemorrhage, and traumatic brain injury. Furthermore, Mg has a wide therapeutic index, and is safe and well-tolerated by patients with acute stroke. Therefore it is not surprising that Mg is the latest potential neuroprotective treatment to be tested in stroke patients. IMAGES (Intravenous Magnesium Efficacy in Stroke) was an international, multicenter, double-blind, placebo-controlled study in acute ischemic stroke. 2589 patients were randomly assigned. However, the main findings were disappointing in that the primary outcome was not improved by Mg. Median time to treatment was 7 h with only 3% of the participants receiving active therapy within 3 h, which may explain the loss of effectiveness of Mg therapy. This time-delay factor should not be a barrier for the FAST-MAG (Field Administration of Stroke Therapy - Magnesium) trial. The purpose of this trial is to demonstrate that paramedic initiation of intravenous Mg sulfate within 2 hours of symptom onset improves the long-term functional outcome of stroke patients. FAST-MAG is about to be launched.

Key words: magnesium, cerebral ischemia, pathophysiology, animal experiments, clinical trials, review

Drug Treatment in Acute Myocardial Infarction – The Position of Magnesium

R. H. Smetana

University Clinic of Vienna, Department of Internal Medicine IV, Vienna, Austria

According to the ACC/AHA guidelines for the management of patients with acute myocardial infarction the recommendation is as follows: antithrombotic and anticoagulant therapy; intravenous nitroglycerin for the first 24-48 hours, prolonged use over 48 hours in case of recurrent angina; reperfusion therapy such as acute PTCA or thrombolysis, in addition to these procedures/agents other routineously applied drugs such as beta-blockers are an established regimen; calcium channel blockers such as verapamil or diltiazem may be given to patients in whom ß-adrenergic receptor blockers are ineffective or contraindicated. In borderline patients the new generation of short-life beta-blockers such as esmolol could also serve as an appropriate substance. The use of intravenous magnesium could be the ideal agent in case that conventional antianginal therapy is not applicable due to aggravation of hypotension. Furthermore, several large trials have shown essential benefit for intravenous magnesium therapy with regard to reduction of life-threatening arrhythmias and left ventricular failure. ACE-inhibitors in the early post-infarction phase are indicated in extended anterior wall infarction and are generally useful. Therefore, the optimal therapy regimen in acute myocardial infarction requires careful titration of antianginal and hypotensive drugs in order to establish equilibrium of pain relief and sufficient myocardial perfusion for the benefit of the patient. Future prospects are focussed on intravenous short-acting betablockers and intravenous magnesium.

Key words: acute myocardial infarction, drug treatment, magnesium

Mutational Analysis of Mrs2 Family of Mg^{2+} Channel Proteins

J. Weghuber, M. Piskacek, R.J. Schweyen

Max Perutz Laboratories, Vienna Biocenter, University of Vienna. Dr. Bohr-Gasse 9, A-1030 Vienna, Austria

Mrs2 proteins are Mg^{2+} channel proteins present in mitochondria of all eukaryotes and in chloroplasts as well as in the plasma membrane of plants. Like their distant homologs CorA in bacteria, they are characterized by two adjacent transmembrane domains near their C-terminus and a motif F/Y-G-M-N in the sequence connecting them. Mitochondrial Mg^{2+} channels are made up of four Mrs2 proteins (= 8 TM domains). Their large N-terminal sequences are oriented towards the inside of the membrane.

Besides the two TM domains Mrs2 proteins share several well conserved sequence blocks, notably two putative coiled-coil domains which are likely to contribute to the oligomerization of Mrs2p. We have introduced a series of mutations in these coiled-coil domains. Surprisingly, most of them dramatically increase Mg^{2+} flux rates and steady state Mg^{2+} levels. As a working model we propose that mutations affecting oligomerization of Mrs2p also influence flux control of the channel. Yeast cells

with these mutations are defective in respiratory growth, which appears to indicate that unphysiologically high Mg^{2+} concentrations in mitochondria have deleterious effects.

Key words: mitochondrial Mg2 + influx, mrs2 (mutant, mag-fura 2, coiled-coil regions

Influence of magnesium and other cations on surface potential of cardiomyocytes

R. Wilhelm[1], V. Beckmann[2], W. Vierling[1]

[1] *Institut für Pharmakologie u. Tox. der TUM, Biedersteiner Str. 29, D-80802 München.*
[2] *NASA Space Flight Center, Washington, USA*

Changes in the extracellular concentrations of divalent cations alter current-voltage relationships (IURs) of ion currents. For example, an increase in $[Mg^{2+}]o$ from 1.2 to 9.6 mM shifts IUR of L-type calcium channels by about 10 mV in guinea pig ventricular cardiomyocytes.
The aim of the present study was a more detailed investigation of the influence of Mg and comparison with the influence of Ca on IUR. Accordingly, $[Mg^{2+}]o$ was varied between 0 and 10 mM and a concentration-effect relationship (CER) for the shift of IUR was established. The resulting relationship could be described by saturation kinetics with a Kd value of about 24 mM Mg. Also the CER of Ca followed saturation kinetics with a Kd value of about 11 mM. These results show that Ca has an about 2-fold stronger effect in comparison to Mg. Also the IUR of Na channels was shifted by increasing the $[Mg^{2+}]o$ but to a lower extent than that of Ca channels.
From the results that both shifts of CERs for Mg and Ca can be described by saturation kinetics but with different Kd values, it can be concluded that divalent cations are not simply physically adsorbed to negative charges at the surface of the cell membrane but that ionic binding plays a role. The different amounts of shifts regarding different ion channels, probably can be explained by differences in the distribution of negative charges in the vicinity of these channels. The alterations of IURs of the channels by Mg may have an influence on Ca influx into the cell and also can explain the "membrane stabilizing" and thereby antiarrhythmic effect of Mg.

Key words: magnesium, calcium inward current, surface potential, heart

Is there a neuroprotective effect of Magnesium? (Results of the IMAGES-study)

K. Wink

University Freiburg, Victor-Kretz-Str. 11, 77723 Gengenbach, Germany

The acute stroke is the third leading cause of mortality in developed countries. Treatment options in the intermediate hours after stroke are limited. Recombinant tissue plasminogen activator (rtPA) greatly improves chances of full recovery if given intravenously within 3 hours of stroke onset but the short time window and bleeding risks restrict rtPA to a few stroke patients. Aspirin has only a small absolute benefit about 1% reduction in death or disability if started within 48 hours of stroke.
Magnesium is neuroprotective in various animal models (e.g. reduction of infarct volume) if given 6 hours after onset of ischaemia. Also workers on several small pilot trials in stroke have reported reduced proportions of magnesium-treated patients being dead or disabled at 3 – 6 months.
In conclusion in the Intravenous Magnesium Efficacy in Stroke (IMAGES) trial was tested the hypothesis wether intravenous magnesium sulphate, given within 12 hours of stroke onset, reduces death or disability at 90 days.
2589 patients were randomised within 12 hours of acute stroke to receive 16 mmol/h MgSO4 intravenously over 15 minutes and then 65 mmol over 24 hours, or matching placebo. Death or disability were not improved by magnesium, but planned subgroup analyses showed benefit of magnesium in non-cortical strokes (p = 0,011).
Magnesium may be of benefit in lacunar strokes.

Key words: magnesium, neuroprotective effect, stroke, clinical study (IMAGES)

Changes in serum magnesium levels during acute and chronic treatment with biopolyphenols in humans

S. Wurzinger, W. Wonisch, R. Wintersteiger, M. Maresch, S. Porta

Institut für Angewandte Stressforschung, Bad Radkersburg, Institut für Pathophysiologie, Graz, Institut für Pharmazeutische Chemie und Pharmazeutische Technologie, Graz, Austria

Introduction, material and methods: High ionized magnesium (Mg) serum levels may either point to stress or even to satisfactory mg status, while

low Mg levels nearly always point to chronic exhaustion. To investigate if this holds also true for oxidative stress, we subdivided a group of 16 male probands out of the Austrian antiterror unit COBRA into a group with higher and a group with lower basal Mg values than average and checked their ionized serum Mg, BE, oLAb and peroxydase.
Results and discussion: Only in the low Mg group significant linear correlations between Mg and oLab respectively peroxidase were evident, which we interpreted as sign of already limited resources. When the same probands have been treated with 300 mg mg and 150 mg biopolyphenols (BPP) per day for 8 days, Mg values nevertheless decreased further, but Mg/peroxidase correlation turned from negative into positive. The same applies to the correlation of Mg/BE product and peroxidase. Persisting low Mg levels may be explained by both BPP-complexation and increased oxidative phosphorylation, while BPP application could activate peroxidase, resulting in reversing the slope of peroxidase.
The role of low Mg levels as indicators for immanent exhaustion seems to be also pertinent concerning oxidative stress management.

Key words: magnesium – changes, oxidative stress, biopolyphenols